Integrating Transcriptomics for the Identification of Potential Age-related Genes and Cells in Three Major Urogenital Cancers Across the Cancer Genome Atlas

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Abstract

Background: Cancer is often defined as a disease of aging. The majority of patients with urogenital cancers are the elderly, whose clinical characteristics are greatly affected by age and aging. Here, we aimed to explore age-related biological changes in three major urogenital cancers by integrative bioinformatics analysis.

Methods: First, mRNA (count format) and clinical data for bladder cancer, prostate cancer and renal cell carcinoma were downloaded from the Cancer Genome Atlas (TCGA) portal. The expressions of 64 cells were obtained by xCell deconvolution method. EdgeR package and limma package were used to analyze differentially expressed genes and cells in the young group and the old group, respectively. ClusterProfiler R package and clueGO plugin were used for enrichment analysis, and cytohubba plugin was used for hub genes analysis. Then co-expression analysis and chromosome distribution for hub genes were analyzed and demonstrated by RIdeogram R package. The clinical correlation of hub genes and key cells was analyzed by Graphpad Prism software. Finally, the correlation between hub genes and key cells was explored by corrplot R package.

Results: We screened and identified 14 hub genes and 4 key cells related to age and urogenital cancers. The age-related differentially expressed genes and co-expressed genes were mainly enriched in muscle movement (Cl^-, Ca^{2+}), inflammatory response, antibacterial humoral immune response, substance metabolism and transport, redox reaction, etc. Most of the age-related genes are on chromosome 17. Moreover, the correlation between cells and genes was analyzed.

Conclusion: Our study analyzed age-related genes and cells in the tumor microenvironment of urogenital cancers, and explored the pathways involved. This could contribute to personalized therapy for patients of different ages and a new understanding of the potential relationship between the aging microenvironment and urogenital cancers.

1 Background

Age is one of the most important risk factors for cancer, and the prognosis of cancer patients is also affected by age of diagnosis [1]. People of all ages can develop tumors, and for most malignancies, the risk for cancer development increases with aging. With the progress of modern medicine, the average life expectancy is increasing, and the incidence of almost all kinds of tumors is increasing year by year [2]. However, patients with different ages and different tumors show unique biological and genomic characteristics, leading to differences in clinical characteristics and treatment outcomes [3]. Most of the existing studies have split the relationship between age and tumor, and there are few studies on the potential mechanism of the relationship between age and tumor. In the era of personalized medicine, the influence of age on tumor cannot be ignored, but there are few studies on the relationship between age and tumor, and the specific mechanism is unclear.

It is well known that the occurrence of tumors is the accumulation of genetic mutations, a process from quantitative to qualitative change. The underlying mechanism in both cancer and aging is the time-dependent accumulation of cellular damage [4]. From this point of view, the high incidence of tumors in the elderly is a normal phenomenon. Studies have shown that half of all tumors occur in people over 70 [5]. Generally speaking, the elderly have a high incidence of tumor and a poor prognosis. But not all is so. Compared with the elderly, most young adults with cancers have a better prognosis and a longer survival period, but some younger patients with tumors have a poor prognosis, such as cholangiocarcinoma, breast cancer and cervical cancer [6–8]. Most of the tumors occurred in the elderly, but leukemia, retinoblastoma, nephroblastoma, and so on, the onset of the peaks are in the juvenile period [9]. Recently, the incidence of some tumors is getting younger, such as colorectal cancer, lung cancer, etc. And the younger these tumors' patients were, the more malignant they were [10, 11]. Even there are studies showing that older patients are less effective at immunotherapy than younger patients [12]. Including above, considerable evidence shows that patients with the same tumor at different ages exhibit different clinical characteristics. These differences and their mechanisms are urgent problems to be studied and solved in clinical work. The study on the relationship between age and tumor will enable us to have a deeper understanding of the occurrence and development of tumor, and to promote the personalized treatment of tumor and optimize patient management.

Cancer is often defined as a disease of aging. For the past few years, putting forward the concept of the aging microenvironment, immunosenescence [13, 14], a growing number of researchers have been paying more attention to the effects of age-related changes in the tumor microenvironment. Aging and cancer are tightly interconnected, and each arm of the tumor microenvironment is altered with aging, contributing to increased tumorigenesis [15]. Different tumor microenvironment in different ages may have different gene mutation patterns and different cell infiltration patterns, which are the main reasons for the differences in some clinical features. These features could also be clinically exploited to develop companion diagnostics and novel therapies for treating different ages patients with cancers [16]. Understanding the contribution of the aging microenvironment to cancer development and progression may lead to better interventions for patients of different ages.

Urogenital cancers account for approximately 14% of human cancers in industrialized countries [17]. Kidney, prostate, and bladder cancers are the three major tumors of the urinary tract, which are among the 10 most frequent cancers in man [2, 18]. Urogenital cancers are mainly in elderly patients, and age has a more prominent influence on the treatment and outcome of tumors. This study aims to explore the influence of age on the prognosis of urogenital cancers by bioinformatics analysis, and to analyze the potential age-related genes and cells in the tumor microenvironment. Finally, we identified 14 hub genes and 4 key cells, and the change of these genes and cells could affect genomic characteristics and clinical characteristics of urogenital cancers due to aging. Furthermore, this study also provides a new clue and approach for other researchers to explore other cancers or diseases related to aging.

2 Materials And Methods

2.1 Data source and pre-processing

The RNA-Seq gene expression profiles (count format) and clinical data of kidney, bladder and prostate cancers were downloaded from the TCGA database using the gdc-client download tool. All statistical extraction and analyses were performed with R software (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism (version 8.0, GraphPad Software, La Jolla, California). After excluding cases with incomplete age and survival data (4 cases of bladder cancer and 3 cases of renal cell carcinoma), patients were divided into the young group and the old group according to the median age. The number of cases included in this study and median age are as follows, bladder cancer 405/409 (cases included /all TCGA cases) (median age value is 69 years), prostate cancer 500/500 (median age is 61 years), renal cell carcinoma 884/887 (median age is 60 years).

2.2 Influence of age on clinical characteristics of urogenital cancers

The Kaplan-Meier analysis for overall survival was proceeded based on the group in the previous step for three kinds of cancers with the aid of GraphPad Prism software and the Log-Rank was utilized to test. Then, the percentages of the major clinical characteristics of the patients in the old/young two groups in the three urogenital cancers were visualized.

2.3 Screening age-related differently expressed genes

The differently expressed genes (DEGs) in the two groups were analyzed via edger R package, and |Log2FC (fold change) | > 1 and $P < 0.05$were set as the screening criteria for DEGs. After getting DEGs of three kinds of urogenital cancers, Venn calculation with online website (https://bioinfogp.cnb.csic.es/tools/venny/) was used to screening the common DEGs.

2.4 Enrichment analysis of the common DEGs

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were used to annotate the structure, functions, and pathways of genes. ClusterProfiler is one of the common tools for enrichment analysis [19]. We made the GO and KEGG pathways analysis via clusterProfiler R package for the common DEGs. Counts ≥ 3 and $P < 0.05$ were set as the enrichment cut-offs to screen meaningful enrichment results. The top 10 terms of biological processes (BP), molecular functions (MF), cellular components (CC) and KEGG were visualized. For the fewer KEGG pathways in the result, the clueGO plugin in Cytoscape software was used to further enrichment analysis for BP and KEGG.

2.5 Protein-protein interaction network construction and hub genes analysis

The 188 DEGs were imported into the STRING database (https://string-db.org/), a web tool used to explore protein-protein interactions (PPI), and the combined-score was set to ≥ 0.4. There are 139 nodes and 317 edges in the PPI network. The network was reconstructed using Cytoscape, and the cytohubba plugin was used to screen the top 30 genes in the network as the central genes using 5 algorithms,

Maximal Clique Centrality (MCC), Density of Maximum Neighborhood Component (DMNC), Maximum Neighborhood Component (MNC), Edge Percolated Component (EPC) and Degree method. The genes obtained by various algorithms were intersected to obtain the hub genes.

2.6 The expression of hub genes and clinical relationship

Univariate Cox regression analysis was used to analyze the prognosis of the hub genes, with covariables set as sex and age. Then the differences in mRNA level and the prognostic of the hub genes in the three urogenital cancers were demonstrated by ggplot2 R package. We used the website cbioportal (www.cbioportal.org/) [20] to analyze the mutations of hub genes in three urogenital cancers, and the cancer selected are Bladder Cancer (TCGA, Cell 2017), Kidney Chromophobe (TCGA, PanCancer Atlas), Kidney Renal Clear Cell Carcinoma (TCGA, PanCancer Atlas), Kidney Renal Papillary Cell Carcinoma (TCGA, PanCancer Atlas) and Prostate Adenocarcinoma (TCGA, PanCancer Atlas). The correlation analysis between hub genes and the clinical characteristics used the WGCNA package.

2.7 Co-expression analysis of hub genes

On the gene level, Guilt by Association means that genes with the same biological effect will change with the change of the expression of other genes [21]. Co-expression analysis is an analysis and interpretation of this phenomenon. The obtained hub genes were analyzed to get the co-expressed genes using PCViz website (www.pathwaycommons.org/pcviz). Then the chromosome distribution of hub genes and co-expression genes was demonstrated by the RIdeogram R package [22]. Metascape website (https://metascape.org/) was used for further enrichment analysis of hub genes and co-expressed genes [23].

2.8 Changes in cells infiltration patterns

The xCell is an algorithm that using deconvolution algorithm to calculate 64 kinds of cells in tissue according to gene expression matrix, including 48 kinds of cells closely related to tumor microenvironment [24]. We downloaded the cell content data of all TCGA samples from xCell website (https://xcell.ucsf.edu/) and extracted the data of urogenital cancers. We used limma R package for differential analysis of cell content. $|logFC| > 1$ and $P < 0.05$ was set as the cut-off, and the difference was demonstrated by volcano map. Veen calculation was used to obtain the cells with different contents in the three tumor microenvironments. Then, univariate cox regression analysis was performed on the relationship between cells content and patient prognosis, and ggplot2 package was used to visualize the results. The association between cells infiltration and clinical characteristics of bladder cancer was analyzed and demonstrated via WGCNA R package.

2.9 The relationship between key cells and hub genes

The corrplot R package was used to conduct correlation analysis of hub genes and key cells to explore the relationship between these genes and cells. At present, the mechanism of age in tumors is not fully understood, and correlation analysis may provide possible biological links between the hub genes and key cells.

3 Results

3.1 Influence of age on clinical characteristics of urogenital cancers

The Kaplan-Meier analysis results of the three urogenital cancers are shown in Fig. 1A-C, in which the prognosis of the elderly patients with bladder cancer and kidney cancer is significantly worse than that of the younger patients, while in the prostate cancer patients, there is no significant difference between the two groups. The reason may be that only 10 of the 500 prostate cancer patients in the TCGA database were observed to die. As shown in Fig. 1D-F, the proportion of patients with T stage and M stage of pathology in the old group of patients with the three types of tumors was higher to the late stage, and the difference in N stage was not significant. In bladder cancer, the old group had a higher proportion of high grade. In prostate cancer, the old group had a higher Gleason stage.

3.2 Screening age-related differently expressed genes

The volcano map (Fig. 2A-C) shows the distribution of DEGs in bladder cancer, prostate cancer and renal cell cancer. The number of DEGs were BC 453/344 (up-regulated/down-regulated), prostate cancer 189/126 and renal cell carcinoma 203/214, respectively. By Venn calculation (Fig. 2D), we selected 188 genes that were DEGs in at least two kinds of cancers. These common DEGs are used for subsequent analysis.

3.3 Enrichment analysis of the common DEGs

The clusterProfiler R package was used for enrichment analysis of the 188 Common DEGs, and the results were shown in Fig. 3A-D. GO analysis results showed that changes in the biological process (Fig. 3A) were mainly enriched in spinal cord development, striated muscle contraction, antimicrobial humoral response and immune response, steroid metabolic process, etc. Changes in the cellular component (Fig. 3B) were mainly enriched in contractile fiber, myofibril, myosin filament, sarcomere, Z disc, I band, myosin complex, blood micro particle, muscle myosin complex, I band, etc. Changes in molecular function (Fig. 3C) were mainly enriched in monooxygenase activity, hormone activity, oxidoreductase activity, substrate-specific channel activity, steroid hydroxylase activity, structural constituent of muscle, oxygen binding, FMN binding, intracellular calcium channel activity, intracellular chloride channel activity, etc. KEGG pathway analysis (Fig. 3D) demonstrated that retinol metabolism, Metabolism of xenobiotic by cytochrome P450.

Since there were fewer enrichment pathways, clueGO was used to conduct enrichment analysis of the involved pathways and biological process again (Fig. 3E), and the results were mainly in musculoskeletal movement, sensory perception of mechanical stimulus, oxidoreductase activity, antimicrobial humoral immune response mediated by antimicrobial peptide, ventral spinal cord interneuron fate commitment, acute phase response, dorsal spinal cord development, intracellular cilium activated chloride channel activity, etc.

3.4 Protein-protein interaction network construction and hub genes analysis

To explore the interrelation of 188 age-related DEGs and obtain hub genes, we made a PPI analysis. Figure 4A-E shows the front 30 hub genes of the five algorithms, namely MCC, DMNC, MNC, EPC, and Degree. Veen operation (Fig. 4F) was conducted on the common genes of the front 30 central genes of five algorithms, and 14 hub genes were obtained. The full names and function of 14 hub genes were shown in Table 1. These genes differ in the tumor microenvironment of patients with urinary tract tumors of different ages, which is likely to influence age-induced clinical characteristics.

Table 1
Functional roles of the 14 hub genes

No.	Gene	Full Name	Function
1	ORM1	Orosomucoid 1	Innate Immune System and Carvedilol Pathway, Pharmacokinetics.
2	ORM2	Orosomucoid 2	Innate Immune System and Response to elevated platelet cytosolic Ca2+.
3	NEUROG3	Neurogenin 3	DNA-binding transcription factor activity and RNA polymerase II proximal promoter sequence-specific DNA binding.
4	INSM1	Insulinoma-associated 1	Regulation of beta-cell development and Developmental Biology.
5	MYBPH	Myosin Binding Protein H	G13 Signaling Pathway and Actin Nucleation by ARP-WASP Complex.
6	TRIM63	Tripartite Motif Containing 63	Ligase activity and obsolete signal transducer activity.
7	MYLPF	Myosin, Light Chain 11, Regulatory	Calcium ion binding and structural constituent of muscle.
8	MYH6	Myosin Heavy Chain 6	Sertoli-Sertoli Cell Junction Dynamics and Translocation of GLUT4 to the plasma membrane.
9	CSRP3	Cysteine And Glycine Rich Protein 3	Structural constituent of muscle and telethonin binding.
10	MYH7	Myosin Heavy Chain 7	Sertoli-Sertoli Cell Junction Dynamics and Translocation of GLUT4 to the plasma membrane.
11	TCAP	Titin-Cap	Ion channel binding and structural constituent of muscle.
12	TNNT3	Troponin T3, Fast Skeletal Type	Actin binding and tropomyosin binding.
13	MYH7B	Myosin Heavy Chain 7B	Myosin Heavy Chain 7B.
14	HP	Haptoglobin	Serine-type endopeptidase activity and hemoglobin binding.

3.5 The expression of hub genes and clinical relationship

Figure 5A shows the differences of 14 hub genes at the mRNA level. Figure 5C shows the mutation frequency of hub genes. Elderly cancer patients tend to have higher mutation frequency due to the accumulation of gene mutations. As shown in Fig. 5C, the mutation frequency of MYH7, MHY6 and MYH7B is higher than other genes, and exceeds 2.5%.

In terms of the clinical correlation of hub genes, Fig. 5B shows the relationship between 14 hub genes and prognosis, and Fig. 5D shows the correlation between hub genes and clinical characteristics of bladder cancer patients. Figure 5B shows that different genes have different prognostic effects on patients with different tumors. From the Fig. 5D, we could find that MYH6 is significantly positively correlated with the age of bladder cancer patients, while MYBPH is negatively correlated with age.

3.6 Co-expression analysis of hub genes

Co-expression analysis can identify genes that change with the expression of the target gene, and these genes often have the same biological process or function. Figure 6A shows the protein-protein interaction network of 14 hub genes and 79 co-expressed genes. The chromosome distribution of co-expressed genes (Fig. 6B) shows that 21 genes were distributed on chromosome 17, accounting for about 22.5%. The chromosome may have some mutations regulating the age-related microenvironment. It is explicit that gene mutation of chromosome 17 is closely related to senile frontotemporal dementia and senile diseases such as Parkinson's disease [25]. Meanwhile, enrichment of the co-expressed genes through Metascape (Fig. 6C-D) showed that the major pathways involved in these genes were in actin, myofibril, skeletal muscle and other muscle motion-related pathways. In addition, interstitial migration, epithelial cell development and other cancer-related pathways are also related to the co-expression genes. These pathways may be involved in age-related epigenetic regulation of urogenital cancers.

3.7 Changes in cells infiltration patterns

The distribution differences of the 48 types of cells in the tumor microenvironment of the three urogenital cancers are shown in Fig. 7A-C. Veen plot (Fig. 7D) showed that there were differences among the four types of cells in the tumor microenvironment, namely Preadipocytes, $CD8^+$ Tem (effect memory, Tem), $CD4^+$T-cells and $CD4^+$ Tem. Preadipocytes increased in the elderly patients' cancer environment and the content of the other three types of immune cells decreased. The relationship between these four types of cells and prognosis is shown in Fig. 7E, in which Preadipocytes are an important prognostic risk factor and the three types of immune cells are prognostic protective factors. The correlation between cells and common clinical traits is shown in Fig. 7F, and Preadipocytes were strongly correlated with the clinical T/N/M stage of bladder cancer patients.

3.8 The relationship between key cells and hub genes

In order to explore the potential biological links between cells and genes, correlation analysis was conducted, and the results are shown in Fig. 8. We can know that there was a strong positive correlation between ORM1, ORM2 and HP, and the three genes showed significantly positive correlation with Preadipocytes. In addition, there was a strong positive correlation between $CD4^+$T-cells and $CD4^+$ Tem. The higher the correlation means the higher likelihood of a potential biological correlation among the cells or genes.

4 Discussion

Kidney, prostate, and bladder cancers increase with age and 82 percent of new cases occurred in individuals aged 60 years and older [26]. The research on the aging microenvironment in tumors is emerging, but the mechanism of its influence on patients of different ages with urogenital cancers is unclear. The development of gene sequencing and bioinformatics provides a feasible approach for us to explore the changes of the aging microenvironment in urogenital cancers from the perspective of bioinformatics analysis. Our study identified 14 central genes, 4 key cells and several potential pathways through bioinformatics analysis of potential and age-related genes and pathway changes in the urogenital cancers' tumor microenvironment. Change of these genes and cells may influence age-induced genomic and clinical differences in urogenital cancers.

Age is one of the major factors affecting the prognosis of patients with urogenital cancers. In this study, elderly patients with bladder cancer and renal cell cancer had poorer overall survival and clinical staging compared with younger patients, which was consistent with other studies [27, 28]. However, there was no significant difference in prognosis between the old group and the young group in prostate cancer. This may be because only 10 deaths were observed in the TCGA database of 500 prostate cancer patients. However, a study of 259 cases of prostate cancer showed increased risk of poor cancer-specific survival in older patients with small-cell Carcinoma of the Prostate [29]. In conclusion, the prognosis in elderly patients with urogenital cancers is poor.

In this study, 14 hub genes were identified and may be the main factor causing differences in overall survival and clinical characteristics of patients of different age. All 14 hub genes and their functions are shown in Table 1. ORM1, ORM2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control [30]. HP has a strong correlation with ORM1 ORM2, and these three genes play a role in many inflammatory diseases, such as sarcoidosis and chronic obstructive pulmonary disease [31, 32]. MYH6, MYH7 and MYH7B are important myosin heavy chain protein, and MYLPF is myosin light chain protein, and MYBPH is myosin binding protein. Mutations in these genes have been linked to various muscle-related diseases and heart disease [33]. CSRP3 is an autophagy regulating molecule that is essential for regulating the degradation of muscle-related components and maintaining normal muscle structure and function [34]. TCAP is a giant elastic protein with kinase activity that extends half the length of a sarcomere and maintains cellular structure [35]. TNNT3 is a fast skeletal muscle Troponin T (TnT), which is involved in initiating muscle contractions [36]. TRIM63 regulates proteasome degradation of cardiac troponin I/TNNI3 and other sarcomeric-associated proteins [37]. INSM1 and NEUROG3 are essential markers for pancreatic neuroendocrine tumors, and related diseases include gastrointestinal tumors, diarrhea and malabsorption [38, 39]. In summary, these hub genes are mainly involved in muscle structure and metabolism, as well as some inflammatory reactions and digestive diseases. Their relationship with aging and tumor needs further study and verification.

The enrichment analysis of age-related differentially expressed genes and co-expressed genes showed that these genes are mainly correlated with muscle structure and contraction regulation, ion channels related to muscle movement (Cl^-, Ca^{2+}), inflammatory response, antibacterial humoral immune response, substance metabolism and transport, redox reaction, etc. And pathways closely related to cancer include

epithelial cell development, nuclear migration, etc. Cancer can be considered as an aging disease. Cancer and aging have many similar biological changes, including changes in intracellular communication, changes in protein stability, metabolic changes and mitochondrial dysfunction, etc. [40]. The biological changes related to age and cancer in this study were mainly changes in muscle activity and various inflammatory responses, which were not completely consistent with previous studies. With aging, myocardial and skeletal muscle movements decrease, and cumulative mutations lead to organ-specific genomic deterioration and dysfunction in old age [41]. Cancer and aging are characterized by similar metabolic disorders, including up-regulation of glycolysis and down-regulation of oxidative phosphorylation [42, 43]. Steroids changes, including adrenocorticosteroid, androgen, estrogen, etc., can promote the occurrence and development of some tumors, and prostate cancer is more relevant [44]. Inflammatory response mainly refers to the changes of immune cells and molecules, and immunosenescence is now closely related to cancer and aging [13, 14]. In all, the relationship between cancer and aging is not completely clear, and the relevant pathways in this study need to be further verified.

There are four cells related to age and tumor formation in the cellular microenvironment, which are Preadipocytes, $CD4^+$ Tem, $CD4^+$ T-cells, and $CD8^+$ Tem. Age significantly affects normal cells in the tumor microenvironment (TME) and promotes tumor progression and metastasis. Fibroblasts and immune cells appear to be particularly vulnerable to this age-related effect [13]. The content of fibroblasts in tumor microenvironment decreased in elderly patients with prostate cancer. Preadipocytes, the only one stromal cell that shows differences in the three types of urogenital cancers, massively increased in elderly patients. The increase of adipose tissue in visceral tissue is one of the hallmarks of aging, which has been linked to cancer, and reduced adipocyte content can extend life [40, 45]. Preadipocytes are closely related to macrophages, and dysdifferentiate in old age, switching into a pro-inflammatory, tissue-remodeling, senescent-like state [45]. Another study shows that this action is achieved through the JAK pathway [46]. For immune cells, $CD4^+$ Tem, $CD4^+$ T-cells and $CD8^+$ Tem were all decreased in elderly patients. It is almost common knowledge that poor immune function in the elderly, also named immunosenescence, can lead to impaired immune surveillance and increased the incidence of the cancer [14]. Immunosenescence causes almost all immune components to change, and CD4 cells and especially CD8 cells are particularly susceptible to immunosenescence [47]. Reversing the expression of these four cells in TME in elderly patients may promote the cure of cancer or alter its clinical characteristics.

In summary, our study identified several age-related genes, cells and pathways in patients with urogenital cancers. These findings can be well combined with age and urogenital cancers, and can be a good guide for further research. Although we have made comprehensive study correlated with tumor microenvironment changes and urogenital cancers, the experiments and clinical validation works with large samples are still necessary.

5 Conclusion

In conclusion, our study analyzed age-related genes and cells in the tumor microenvironment of urogenital cancers, identified 14 genes and 4 cells, and explored the pathways involved. These cells and genes are associated with age-induced differences in clinical characteristics of urogenital cancers. Further study of these cells and genes could contribute to personalized therapy for patients of different ages and a new understanding of the potential relationship between the aging microenvironment and urogenital cancers. Moreover, this study also has some implications for other tumors and age-related diseases.

Declarations

Authors' contributions

The study conception and design were performed by Jinlong Cao and Junqiang Tian. Material preparation, data collection and analysis were performed by Jinlong Cao, Jianpeng Li, Xin Yang, , Pan Li and Zhiqiang Yao. The first draft of the manuscript was written by Jinlong Cao, Jianpeng Li, and Dali Han, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Publicly available datasets were analyzed in this study, these can be found in The Cancer Genome Atlas (https://portal.gdc.cancer.gov/).

Competing interests

The authors declare that they have no competing interests.

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Figures

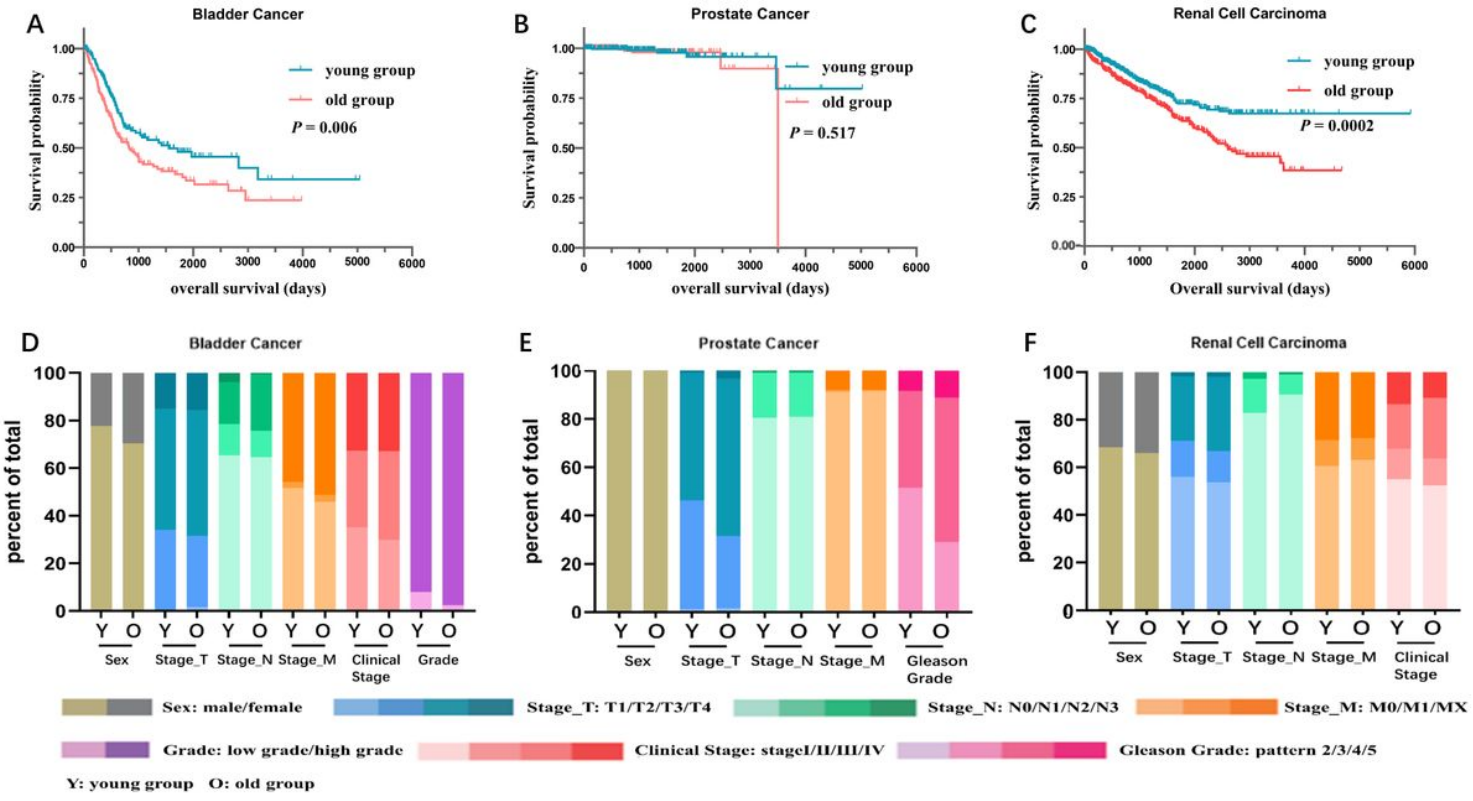

Figure 1

Influence of age on the clinical characteristics of urogenital cancers. (A-C) The survival analysis for the old group and the young group of urogenital cancers. The red line indicates the old group, and the blue line indicates the young group. (D-F) The proportion of main clinical characteristics in old group and young group of bladder cancer, prostate cancer and renal cell carcinoma. Each column of the stacked bar chart represents different groups, and each column with a different color and height indicates the corresponding percent of different clinical characteristics.

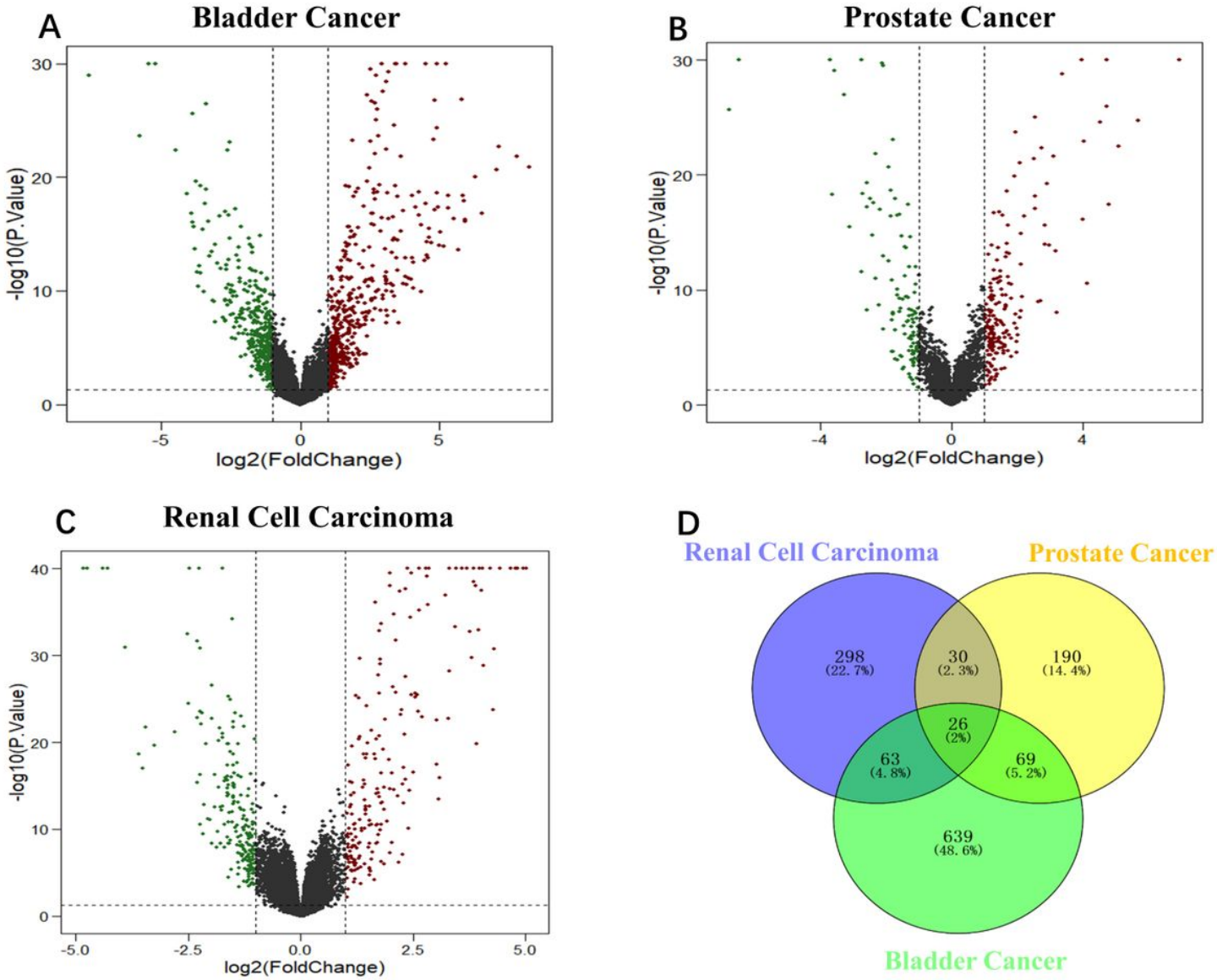

Figure 2

Screening age-related differently expressed genes. (A-C) Volcano plots of the three kinds of urogenital cancers gene expression profiles grouping by age. Red/blue symbols classify the upregulated/downregulated genes according to the criteria: |log2FC (fold change) | > 1 and P-value < 0.05. (D) Venn calculation with online website (https://bioinfogp.cnb.csic.es/tools/venny/) was used for screening and obtaining the common DEGs. We selected 188 genes that were DEGs in at least two kinds of cancers.

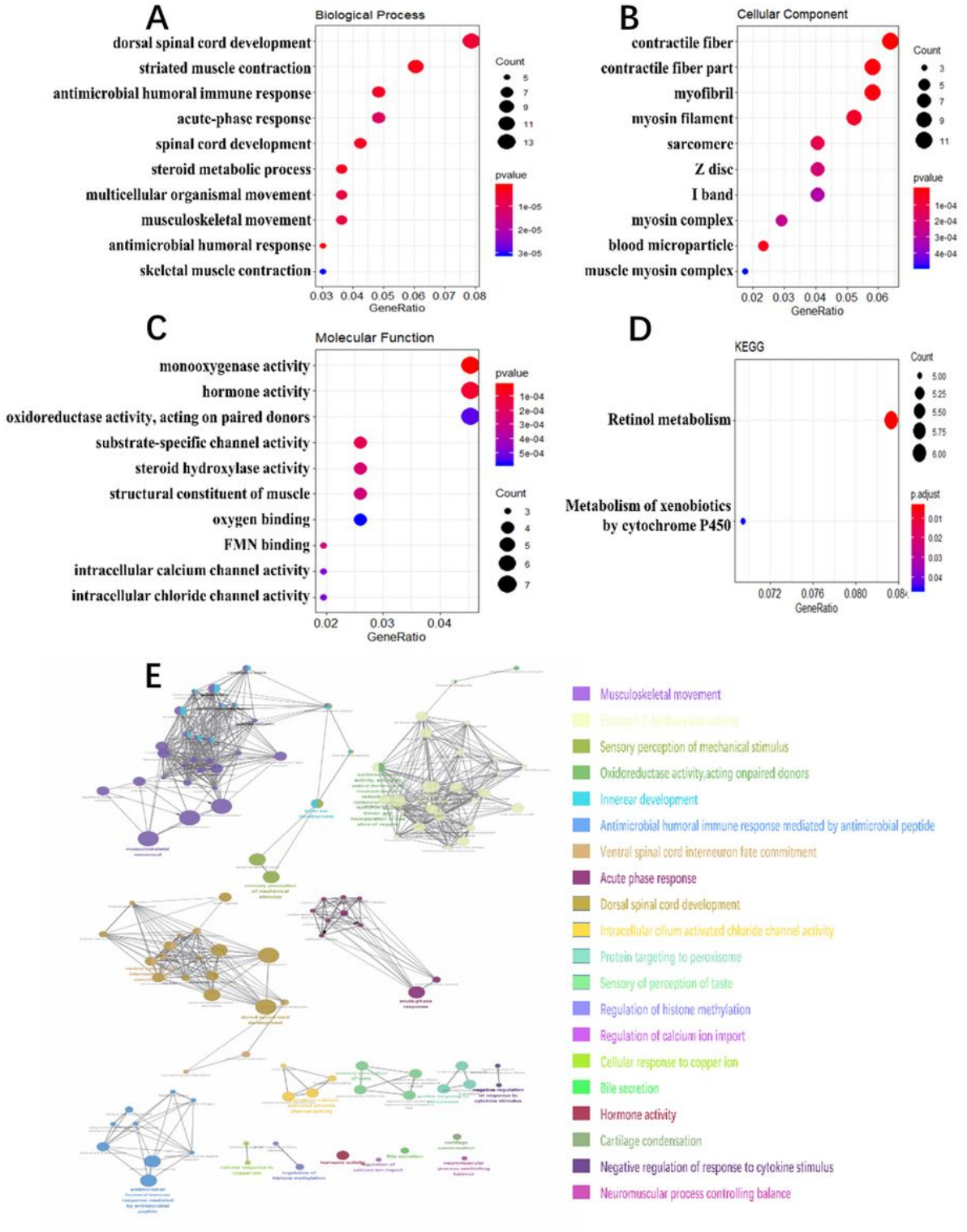

Figure 3

Enrichment analysis of the common DEGs. (A-D) The enrichment analysis results of the common DEGs via clusterProfiler R package, namely biological processes, cellular components, molecular functions, and KEGG. The main 10 results of each term are shown, and the color indicates the significant degree P value of enrichment and the size indicates the number of genes enriched for each result. (E) The enrichment

analysis results of the common DEGs via clueGO plugin in Cytoscape software. Each point represents a gene, and different colors represent different enriched terms.

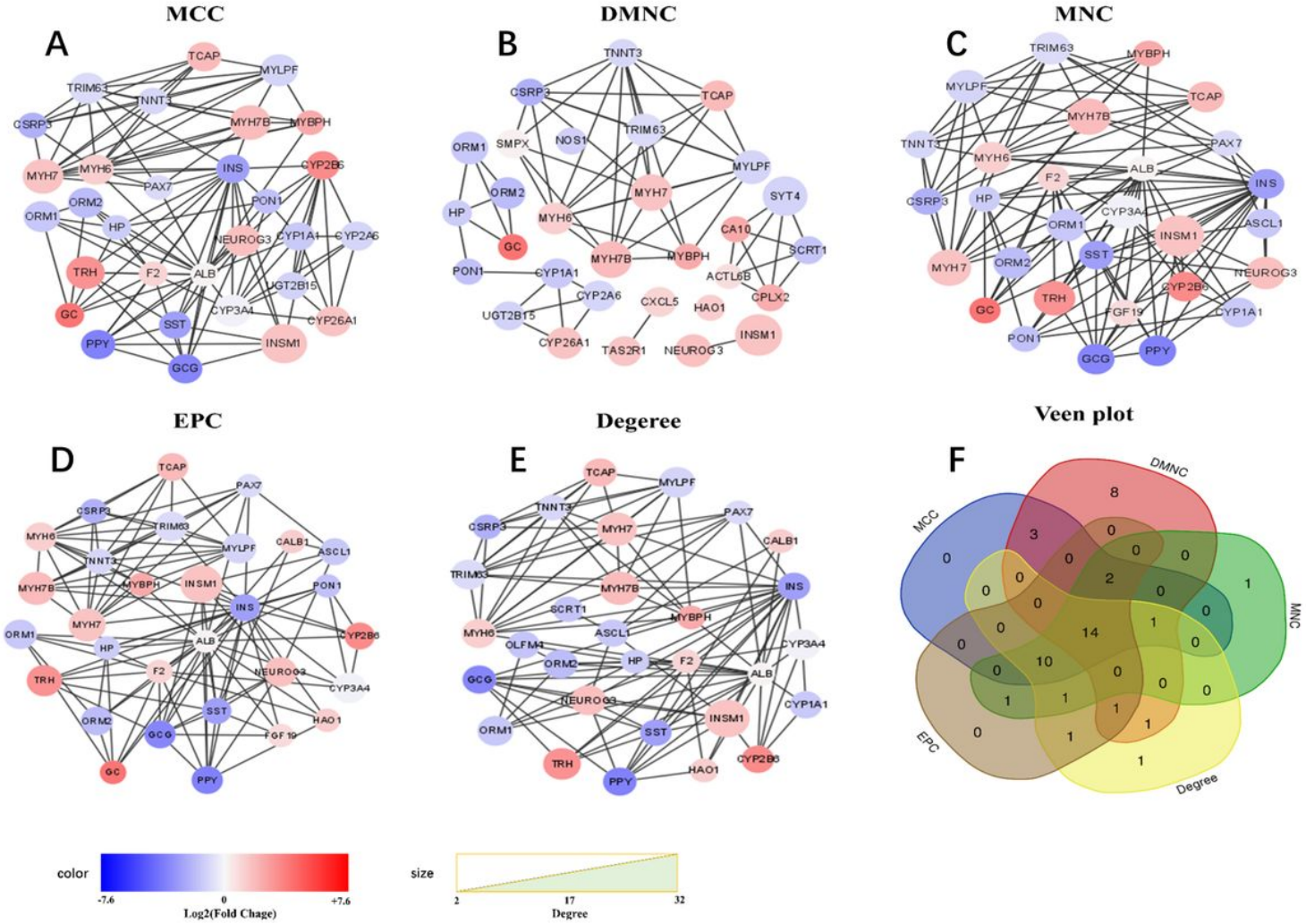

Figure 4

Protein-protein interaction network construction and hub genes analysis. (A-E) Screening the top 30 hub genes in the protein-protein interaction network using 5 algorithms including MCC, DMNC, MNC, EPC, and Degree. The color of the nodes in the protein-protein interaction network reflects the |log2FC (fold change)|, and the size of the nodes represents the number of proteins interacting with each other. (F) Various algorithms obtain a Venn diagram of a common hub genes, and there are 14 common hub genes.

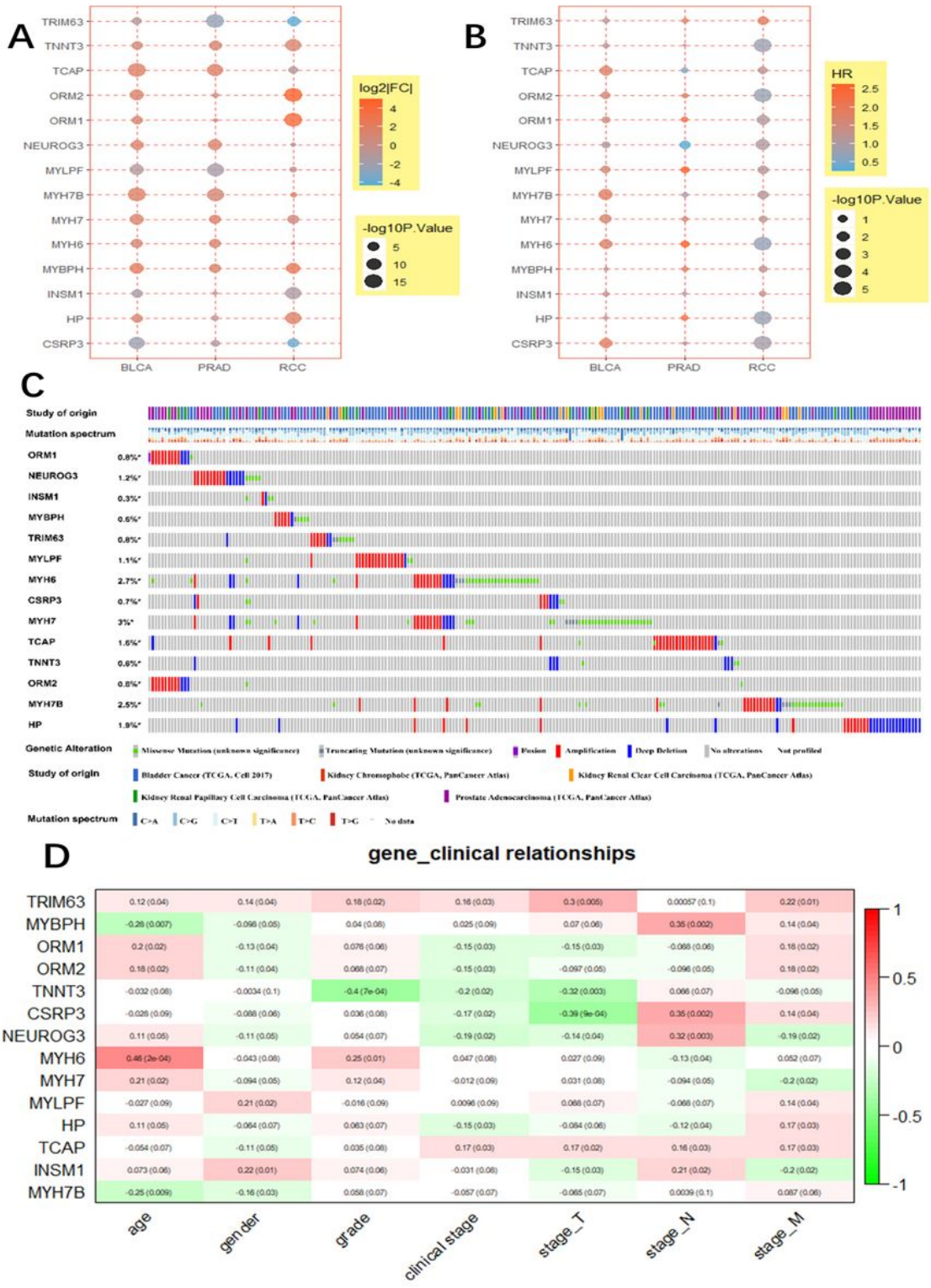

Figure 5

The expression of hub genes and clinical relationship. (A) Expression differences of 14 hub genes at mRNA level. The color of each point represents |log2FC|, and the size represents P value. (B) The results of univariate cox regression analysis of 14 hub genes and overall survival. The color represents HR value and the size represents P value. (C) Mutations of 14 hub genes in urogenital cancers. The figure summarizes genomic mutations in all 14 hub genes across urogenital cancers' sample set. Each row

represents a gene, and each column represents a tumor sample. Red bars indicate gene amplifications, blue bars are deep deletions, green squares are missense mutations, and grey bars are truncation mutations. (D) The correlation between the 14 hub genes and clinical characteristics in bladder cancer. The former numbers in each small rectangle indicate the correlation and the numbers in brackets indicate the P-value for the correlation.

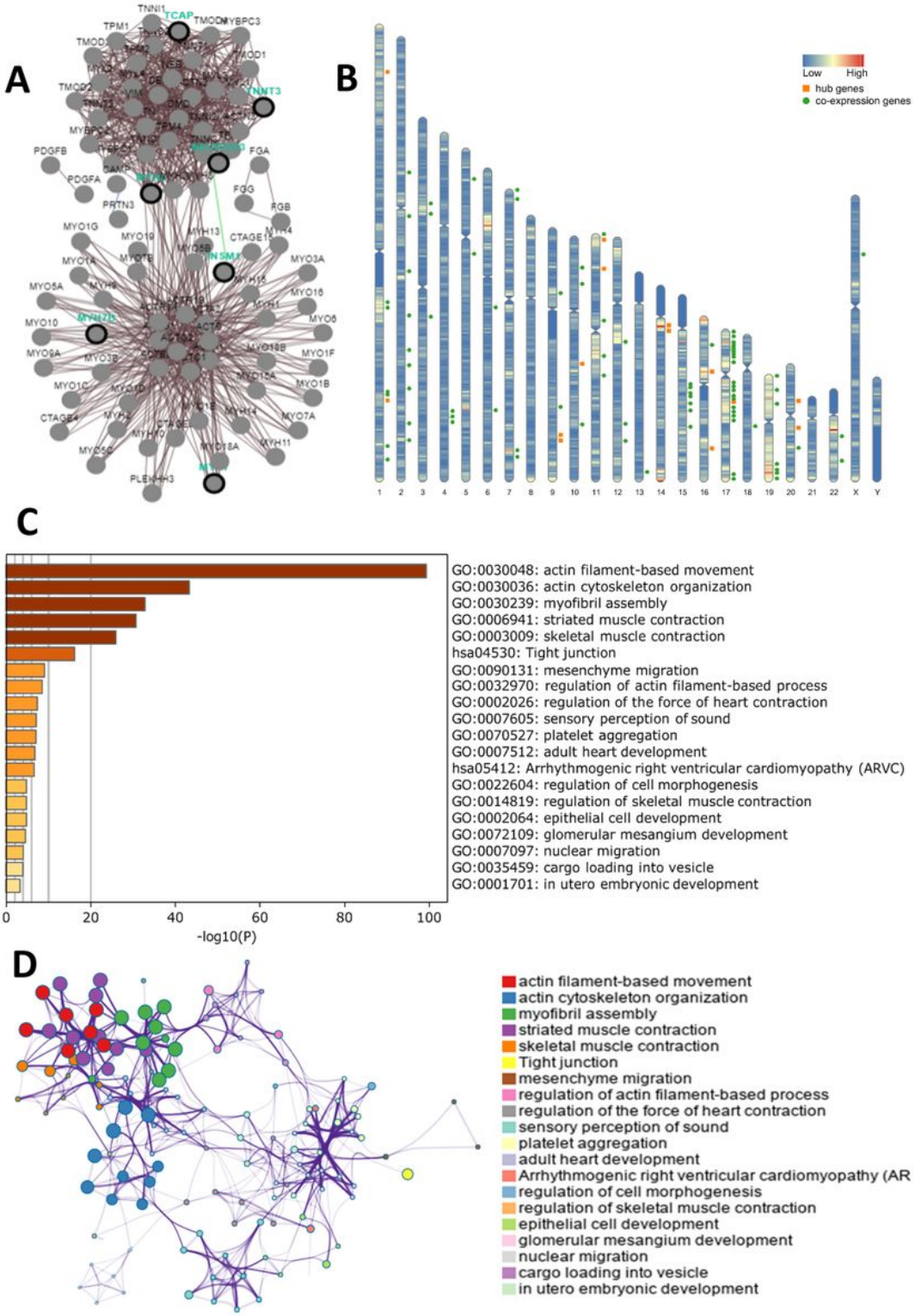

Figure 6

Co-expression analysis of 14 hub genes. (A) The co-expression network was obtained via PCViz online website (www.pathwaycommons.org/pcviz). (B) Distribution of 14 hub genes and 79 co-expression genes on chromosomes. The orange square is hub genes, while the green circle is co-expression genes. (C) Bar graph of enriched terms across the hub and co-expression genes, colored by P-values. (D) The enrichment results were colored by cluster-ID, where nodes that share the same cluster ID are typically close to each other.

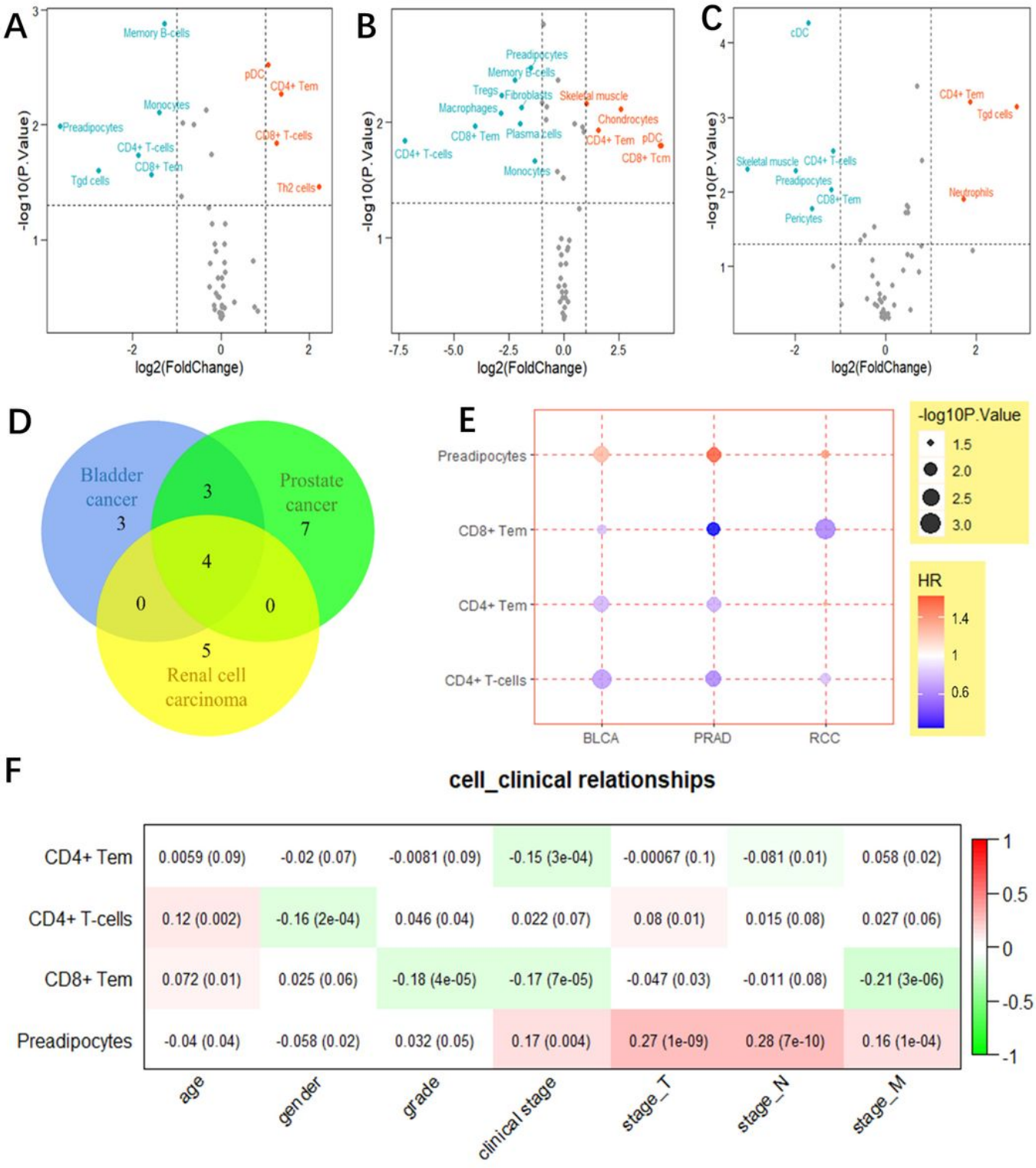

Figure 7

Changes in cells infiltration patterns. (A-C) Volcano maps show the differences of the 48 important cells in the tumor microenvironment by the xCell method. The up-regulated cells in the old group are shown in red, and the down-regulated cells in the old group are shown in light blue. (D) Veen map shows the common different cells in the three urogenital cancers. (E) The results of univariate cox regression analysis of 4 key cells for overall survival. The color represents HR value and the size represents P value. (F) The correlation between the 4 key cells and clinical characteristics in bladder cancer. The former numbers in each small rectangle indicate the correlation and the numbers in brackets indicate the P-value for the correlation.

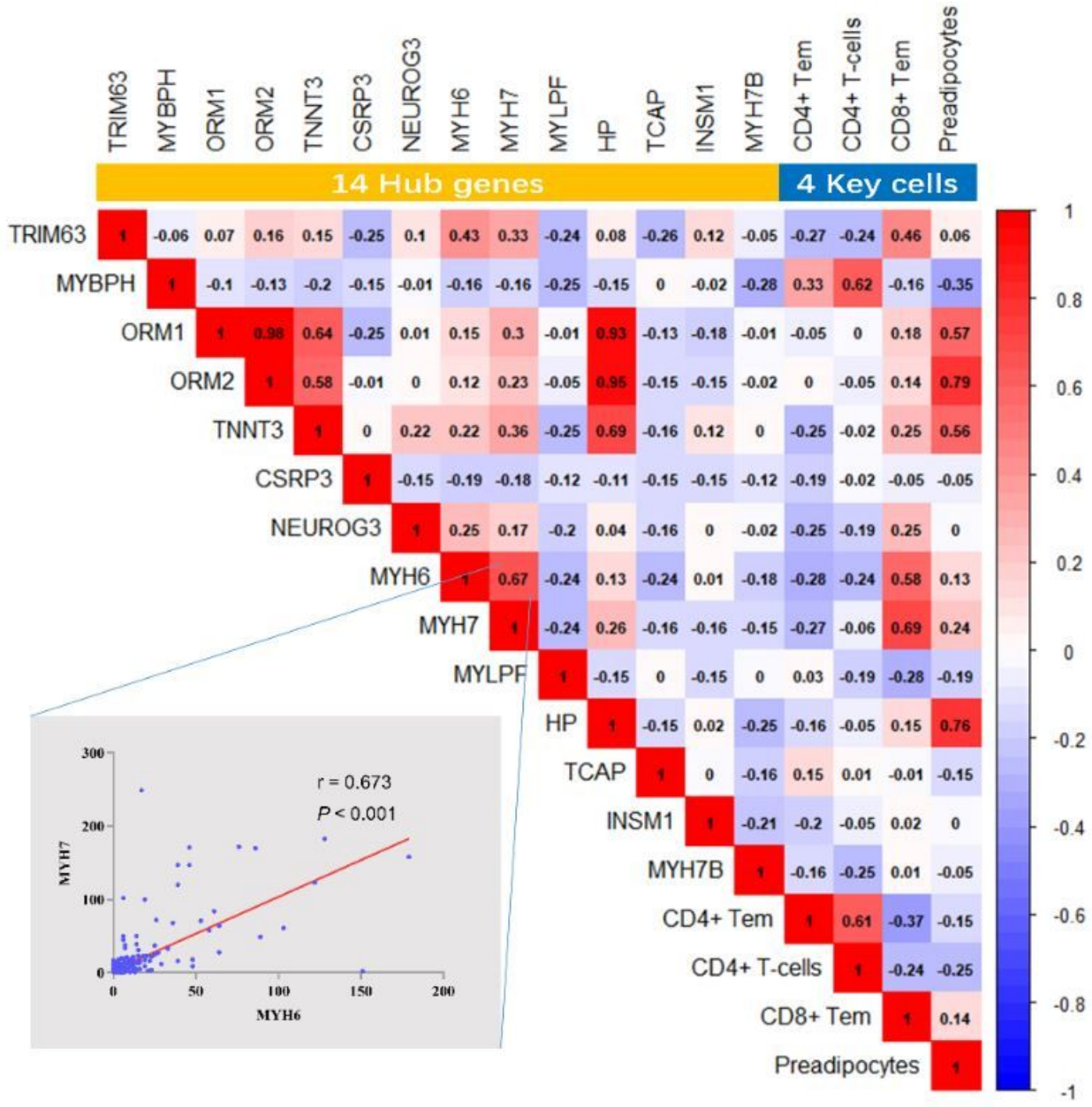

Figure 8

The relationship between 4 key cells and 14 hub genes in bladder cancer. Correlation analysis of expression of 14 central genes and 4 key cells in bladder cancer patients was demonstrated, and the

colors of the square represent the correlation coefficient (Pearson correlation). The correlation map at the bottom left shows the correlation between MYH6 and MYH7.